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Rare sugar D-allulose: Potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus

Akram Hossain[a,], Fuminori Yamaguchi[a], Tatsuhiro Matsuo[b], Ikuko Tsukamoto[c], Yukiyasu Toyoda[d], Masahiro Ogawa[e], Yasuo Nagata[f], Masaaki Tokuda[a,*]

[a] *Department of Cell Physiology, Faculty of Medicine, Kagawa University, 1750-1, Ikenobe, Miki, Kita, Kagawa 761-0793, Japan*

[b] *Faculty of Agriculture, Kagawa University, Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0795, Japan*

[c] *Department of Pharmacobioinformatics, Faculty of Medicine, Kagawa University, 1750-1, Ikenobe, Miki, Kita, Kagawa 761-0793, Japan*

[d] *Department of Pathobiochemistry, Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya Aichi, Japan*

[e] *Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki, Kagawa, 76100795, Japan*

[f] *Department of Nutrition, University of Nagasaki, Siebold, 1-1-1 Manabino, Nagayo-cho, Nishisonogi-gun, Nagasaki 859-2195, Japan*

*Corresponding author: Masaaki Tokuda, Department of Cell Physiology, Faculty of Medicine, Kagawa University, 1750-1, Ikenobe, Miki, Kita, Kagawa 761-0793, Japan. Tel.: +81-87-891-2195; Fax: +81-87-891-2096.

E-mail address: tokuda@med.kagawa-u.ac.jp (M. Tokuda)

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Keywords: Rare sugar, D-allulose, pharmacokinetics, insulin resistance, adiposity, β-cell preservation, therapeutic monitoring, functional food

Abbreviations: T2DM, type 2 diabetes mellitus; OLETF, Otsuka Long-Evans Tokushima Fatty; LPL, lipoprotein lipase; GLUT5, glucose transporter 5; GLUT2, glucose transporter 2; GRAS, Generally Recognized as Safe; FDA, food and drug administration; OGTT, oral glucose tolerance test; GK, glucokinase; GKRP, glucokinase regulatory protein; β-cell, beta-cell; IL-6, interleukin-6; IL-1β, interleukin-1 beta; TNFα, tumour necrosis factor-alpha; SGLT1, sodium-dependent glucose transporter 1; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin-sensitivity check index; CHE, cholinesterase; LDH, lactatedehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; HbA1c, hemoglobin A1c; α-SMA, alpha smooth muscle actin; ROS, reactive oxygen species; TNF-α, tumour necrosis factor-α; MCP-1, monocyte chemoattractant protein 1; SR-B1, scavenger receptor class B type 1; MTP, microsomal triglyceride transfer protein; TG, triglyceride; HFCS, high fructose corn syrup; NAS, non-caloric artificial sweeteners; WHO, World Health Organization

Abstract

Obesity and type 2 diabetes mellitus (T2DM) are the leading worldwide risk factors for mortality. The inextricably interlinked pathological progression from excessive weight gain, obesity, and hyperglycemia to T2DM, usually commencing from obesity, typically originates from overconsumption of sugar and high-fat diets. Although most patients require medications, T2DM is manageable or even preventable with consumption of low-calorie diet and maintaining body weight. Medicines like insulin, metformin, and thiazolidinediones that improve glycemic control; however, these are associated with weight gain, high blood pressure and dyslipidemia. These situations warrant attentive consideration of the role of balanced foods. Recently, we have discovered advantages of a rare sugar, D-allulose, a zero-calorie functional sweetener having strong anti-hyperlipidemic and anti-hyperglycemic effects. Study revealed that after oral administration in rats D-allulose readily entered the blood stream and was eliminated into urine within 24 hours. Cell culture study showed that D-allulose enters into and leaves the intestinal enterocytes via glucose transporters GLUT5 and GLUT2, respectively. In addition to D-allulose's short-term effects, characterization of long-term effects has been focused on preventing commencement and progression of T2DM in diabetic rats. Human trials showed that D-allulose attenuates postprandial glucose levels in healthy subjects and in borderline diabetic subjects. The anti-hyperlipidemic effect of D-allulose, combined with its anti-inflammatory actions on adipocytes is beneficial for prevention of both obesity and atherosclerosis, and is accompanied by improvements in insulin resistance and impaired glucose tolerance. Therefore, this review presents brief discussions focusing on physiological functions and potential benefits of D-allulose on obesity and T2DM.

Contents

1. Introduction

Currently, the incidence and prevalence of excessive weight gain followed by obesity has dramatically increased throughout the world, with the consequence that an estimated 325 million diabetes sufferers will exist during the next 25 years (Wild et al., 2004). Over consumption of sugar and high-fat diets are considered the main causative dietary factors of this situation (Giugliano and Esposito, 2008). Beyond the availability of a number of pharmacological and surgical treatments, lifestyle modifications (Asif, 2014) involving the consumption of foods with low energy density in addition to increasing physical activities are the basic therapeutic strategies to prevent the development of type 2 diabetes mellitus (T2DM).

Recently, we have been studying rare sugars that are defined as "monosaccharides and their derivatives that are rare in nature" by the International Society of Rare Sugars (https://sites.google.com/site/raresugars/). There are more than 50 kinds of rare sugar. One of them, D-allulose (previously named D-psicose), has been determined to have a low degree of energy density, exhibiting almost zero calories (Matsuo et al., 2002a), and thus has been proven to be a unique metabolic regulator of glucose and fat metabolism in a number of basic research (Matsuo and Izumori, 2006; Matsuo and Izumori, 2009; Hossain et al., 2011; Hossain et al., 2012) and clinical (Iida et al., 2013) studies. D-Allulose has demonstrated activity involving a variety of mechanisms, such as strong anti-oxidative effects, inhibitory activity toward intestinal digestive enzymes, enhanced translocation of glucokinase (GK) from the hepatic nucleus to cytoplasm, and competitive transport with glucose through the intestinal mucosa. Therefore, this review will summarize the physical properties, absorption, excretion, and physiological functions of D-allulose, as well as the potential benefits of D-allulose on obesity and T2DM with its safety and possible use as a substitute for conventional sugars.

D-Allulose is a monosaccharide with a molecular formula $C_6H_{12}O_6$. It is a C-3 epimer of D-fructose (Fig. 1) and its systematic name is D-ribo-2-hexulose. D-Allulose is also called D-psicose and the name 'psicose' is derived from the antibiotic psicofuranine, from which it was isolated (Eble et al., 1959). D-Allulose is rarely encountered in nature as a component of some plants, such as *Itea* plants (Zuina) (Poonperm et al., 2007), and certain bacteria (Zhang et al., 2009), but not in higher animals. D-Allulose contains one ketone group and acts as a reducing agent. It is prepared as a white, odorless powder and is easily dissolved in water. The sweetness of D-allulose is about 70% of sucrose, melts at 90°C, and forms caramel. As a reducing sugar, heating with amino acids, peptides, and proteins in foods induces the amino-carbonyl reaction (Maillard reaction) at a lower degree than D-glucose or D-fructose (Sun et al., 2004). These Maillard products show antioxidative activity and gelling properties, such as enhanced gel strength and water-holding capacity (Sun et al., 2006).

Although it is rarely found in nature, it has been reported that commercial mixtures of D-glucose and D-fructose obtained from the hydrolysis of sucrose or D-glucose isomerization (Cree and Perlin, 1968), as well as processed cane and beet molasses (Binkley and Wolfrom, 1953; Thacker and Toyoda, 2009), contain a small quantity of D-allulose as a result of the heating process during manufacturing. After the discovery of the key enzyme D-tagatose 3-epimerase, which converts D-fructose to D-allulose, mass production began (Takeshita et al., 2000; Izumori, 2006). Currently, D-allulose is also produced by chemical synthesis and is widely available at a much lower cost. Thus various D-allulose-added foods have been prepared and marketed in Japan. D-Allulose was approved as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA) in June 2014 (GRAS Notice No. GRN 498), and is allowed to be used as an ingredient in a variety of foods and dietary supplements (Mu et al., 2012).

In addition to its use as a substitute sweetener, D-allulose was also reported to inhibit trichomonad growth by reinforcing the action of metronidazole (Harada et al., 2012), and to induce the up-regulation of defense-related genes in plant cultivation (Kano et al., 2011). Further research into its use as an anti-parasitic or herbicide is currently being undertaken.

2. Absorption, metabolism, and organ distribution of D-allulose

^{14}C-Labelled D-allulose was enzymatically synthesized (Morimoto et al., 2006) to study its absorption, distribution, and elimination after both intravenous and oral administration in Wister rats and the concentrations in whole blood, urine, and organs were measured. D-Allulose (100 mg/kg) was orally administered to rats that were subsequently sacrificed 10, 30, 60, and 120 minutes after administration. The concentrations of D-allulose in urine were 19% and 37% of the administered dose at 60 and 120 minutes, respectively, and almost 0% 7 days thereafter (Tsukamoto et al., 2014). Other studies detected 11% - 15% after 24 - 48 hours (Matsuo et al. 2003) and 35.4% after 7 hours (Whistler et al., 1974). We and others have reported that ≈70% of D-allulose was absorbed and excreted via urine (Whistler et al., 1974; Tsukamoto et al., 2014). However, a small portion of D-allulose was not absorbed in the small intestine, was conveyed to the large intestine, and ultimately found to be partly fermented in the appendix in rats (Matsuo et al., 2003), and to a lesser degree in humans (Iida et al., 2010).

Following intravenous administration, the blood concentration was decreased with a half-life of 57 minutes and excretion in urine was approximately 45% and 50% within 1 and 2 hours, respectively (Tsukamoto et al., 2014). Autoradiography detected high levels of ^{14}C-labelled D-allulose in the liver, kidney, and urinary bladder; interestingly, however, no accumulation was observed in the brain (Tsukamoto et al., 2014).

3. Effect of D-allulose on sugar absorption through rat intestinal mucosa

Sugar absorption occurs primarily in the small intestine. It has been shown that D-allulose is also absorbed from the small intestine and released into the bloodstream (Iida et al., 2010; Tsukamoto et al., 2014). Monosaccharide uptake into enterocytes of the human intestinal lumen is mediated by a sugar transporter located on the apical membrane (brush border membrane), while efflux from enterocytes to the lamina propria is mediated by a different sugar transporter located in the basolateral membrane. In the brush border of enterocytes, two transporters are involved in the uptake of sugars. Uptake of D-glucose is mediated by the active transporter sodium-dependent glucose co-transporter 1 (SGLT1). The energy required for uphill sugar transport is provided by the sodium electrochemical potential gradient, which is maintained by the Na^+/K^+ pump located on the basolateral membrane (Fig. 2). In contrast to D-glucose, D-allulose enters enterocytes by the passive transporter GLUT5, known as the D-fructose transporter (Hishiike et al., 2013). GLUT5 is the only GLUT protein with a high specificity for D-fructose (Douard and Ferraris, 2008), and it is of great interest that GLUT5 is capable of transporting D-allulose. The principal site of GLUT5 expression is the brush border membrane of absorptive enterocytes, where it provides a major route for the absorption of dietary fructose. The predominant sugar efflux transporter in the basolateral membrane of enterocytes is the facilitated diffusion glucose transporter 2 (GLUT2). GLUT2 carries out the downhill transport of various hexoses including D-glucose, D-galactose, D-mannose, and D-fructose. It has been shown that D-allulose is also effluxed through GLUT2 (Hishiike et al., 2013). Because GLUT2 is expressed at a very high level in pancreatic β-cells, as well as in the basolateral membranes of intestinal and kidney epithelial cells and hepatocytes, the incorporation of D-allulose into those cells is likely to be mediated by GLUT2 (Thorens et al., 1993). To date, only the two sugar transporters, that is, GLUT5 and GLUT2, have been identified as carriers of D-allulose (Hishiike et al., 2013). These

transporters are expressed in a variety of tissues and cells other than the small intestine, indicating that D-allulose should be incorporated through GLUT5 and GLUT2 into various tissues and cells.

Experiments aimed at determining the effect of D-allulose on the absorption of D-glucose and D-fructose through the Caco-2 monolayer cell lines were performed (Hishiike et al., 2013). The Caco-2 cell line is derived from human colon adenocarcinoma, and can spontaneously differentiate into small intestinal epithelial cells with a highly functionalized epithelial barrier composed of tight junctions, microvilli, brush border enzymes, and transporters (Bohets et al., 2001; Sambuy et al., 2005; Manzano, 2010). Thus, these cells have been widely used in the assessment of nutrient absorption. We added three monosaccharides (30 mM each of D-glucose, D-fructose, and D-allulose) onto the apical side of a Caco-2 monolayer. The amount of three sugars permeating through the Caco-2 monolayer increased linearly with incubation time. The permeation level of D-allulose after 120 min was low compared with those of D-glucose and D-fructose, with 60% D-fructose-permeation and only 10% of D-glucose-permeation. Thus, it was suggested that the absorption rate of D-allulose from the intestinal lumen is lower than that of the nutritive sweeteners, especially D-glucose. The permeation rate of D-glucose is suppressed in the presence of other sugars. The presence of 30 mM D-fructose or 30 mM D-allulose along with 30 mM D-glucose resulted in 56% and 60% reductions in D-glucose permeation rates, respectively. The simultaneous addition of the three sugars onto the Caco-2 monolayer induces further reduction (70% reduction in D-glucose permeation rate). During influx, D-glucose uses a different sugar transporter than D-fructose and D-allulose. In contrast, during efflux, D-glucose shares the transporter GLUT2 with D-fructose and D-allulose. As a result, it is concluded that the three sugars compete with one another for transport through the basolateral membrane transporter GLUT2. The decreased D-glucose permeability in the presence of D-fructose and/or D-allulose can be

ascribed to the competitive sugar efflux at the GLUT2 transporter on the Caco-2 basolateral membrane.

The permeation of D-fructose is also suppressed by D-allulose or D-glucose in a similar manner. The presence of 30 mM D-allulose or D-glucose with 30 mM D-fructose caused a 50% decrease in the D-fructose permeation rate. The presence of all three sugars induced a 70% reduction in D-fructose permeation rate. The decreased D-fructose permeation rates imply that the efflux transport of the three sugars occurred competitively at the basolateral transporter GLUT2. Furthermore, D-allulose shares the influx transporter GLUT5, located at the brush border membrane, with D-fructose. Therefore, competition between D-fructose and D-allulose might occur in transport across the brush border membrane. However, the suppressive effect of D-glucose on D-fructose permeation was almost the same as that of D-allulose. This indicates that the rate-limiting sugar transporter in the Caco-2 monolayer is GLUT2 in the basolateral membrane. Thus, D-allulose can competitively inhibit the transport of D-glucose and D-fructose at basolateral GLUT2, possibly resulting in decreased absorption of dietary D-glucose and D-fructose in the digestive tract. GLUT2 and GLUT5 are distributed in the intestine, as well as in other tissues and cells. GLUT 2 is mainly expressed in the basolateral membrane of hepatocytes, kidney, small intestine, and insulin-producing β cells (Roncero et al., 2004), while GLUT 5 is expressed in the kidney, fat, skeletal muscle, brain, and sperm (Sasaki et al., 2004; Funari et al., 2005). D-Allulose might partially inhibit the uptake of D-glucose and/or D-fructose at GLUT2 or GLUT5 in those tissues and cells. The suppressive effect of the non-metabolized sugar D-allulose on the uptake of D-glucose and D-fructose might contribute to the important biological functions of D-allulose, such as the improvement of insulin resistance and the inhibition of body fat accumulation (Hossain et al., 2011; Ochiai et al., 2013).

4. Translocation of D-allulose-induces hepatic glucokinase to enhance hepatic glucose utilization

Hepatic D-glucose metabolism is regulated via the shuttling of glucokinase (GK) between the nucleus and cytoplasm (Fig. 3). Glucokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) catalyzes the phosphorylation of D-glucose to glucose-6-phosphate, which is the rate-limiting step in glycolysis, and maintains glucose homeostasis (Matschinsky, 2009). This enzyme is expressed mainly in pancreatic β cells and hepatocytes, and is also present in certain hypothalamic neurons and enteroendocrine cells (Iynedjian, 2009). In the liver, GK regulates both D-glucose uptake (glycogen storage and glycolysis) and D-glucose output through the futile cycle between D-glucose and D-glucose 6-phosphate (Cherrington, 1999). Studies on GK mutations (Vionnet et al., 1992), transgenic animals (Efrat et al., 1994; Grupe et al., 1995; Rossetti et al., 1997; Postic et al., 1999), and patients with T2DM (Mevorach et al., 1998; Basu et al., 2001) have shown that lowered functioning of hepatic GK contributes to the pathogenesis of hyperglycemia in diabetes mellitus. GK is considered a candidate target for anti-diabetic drugs for T2DM (Lloyd et al., 2013).

Immunohistochemical studies have clearly shown for the first time that GK is localized in the nucleus during the postabsorptive state (Miwa et al., 1990), and translocated from the nucleus to the cytoplasm in response to external stimuli (Toyoda et al., 1994; Toyoda et al., 1995), such as altering the D-glucose concentration and/or the presence of small amounts of D-fructose. The mechanism whereby hepatic D-glucose metabolism is regulated by translocation of GK between the nucleus and the cytoplasm is as follows: at low D-glucose concentrations, GK in the nucleus is inactive due to binding to the glucokinase regulatory protein (GKRP); in the presence of high D-glucose concentrations or the presence of low concentrations of D-fructose, which is converted to D-fructose 1-phosphate by ketohexokinase

(KHK), GK dissociates from GKRP and enables GK to be translocated to the cytoplasm. After translocation of GK from the nucleus to the cytoplasm, the free and active form of GK phosphorylates D-glucose to D-glucose 6-phosphate, thereby resulting in rapid glucose utilization for glycogen synthesis and suppression of D-glucose output. Studies have shown that diabetic animals display increased activities of glucose-6-phosphate dehydrogenase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). Both of these enzymes regulate the rate-limiting steps in hepatic gluconeogenic flux and therefore contribute to hyperglycemia in diabetes (Herling et al., 1998). Feeding a D-allulose-containing diet to rats resulted in significantly reduced G6Pase activity, thereby controlling blood glucose levels and exerting hypolipidemic affects (Nagata et al. 2015), although the effect on PEPCK has yet to be evaluated.

T2DM is associated with defective regulation of hepatic glucose metabolism, involving elevated D-glucose production and subnormal postprandial clearance of D-glucose by the liver. This is due to delayed suppression of hepatic D-glucose production and impaired conversion of D-glucose to glycogen. Toyoda and co-workers have found that GK translocation is impaired in the liver of animal models of T2DM, namely OLETF rats (Hossain et al., 2011) and Goto-Kakizaki rats (Toyoda et al., 2000). Impairment of hepatic GK translocation was also observed in Zucker Diabetic Fatty rats (Fujimoto et al., 2004), and diet-induced diabetic rats (Sakamoto et al., 2012) and dogs (Coate et al., 2013). Impairment of GK translocation is involved in both the suppression of hepatic glucose utilization (glycogen storage and glycolysis) and in the acceleration of hepatic glucose output in diabetic rats, thereby contributing to hyperglycemia. Therefore, hyperglycemia in patients with T2DM would be improved by stimulating translocation of GK out of the nucleus, which might represent a new approach to the normalization of hyperglycemia in T2DM (Toyoda et al., 2000; Shiota et al., 2002; Watford, 2002). Decreased hepatic GK activity has been reported in people with

impaired glucose tolerance and impaired fasting glucose levels (Perreault et al., 2014), as well as in patients with T2DM (Caro et al., 1995; Mevorach et al., 1998; Basu et al., 2000; Basu et al., 2001). Therefore, the increase in active GK following translocation (moderate activation of GK) could be sufficient to improve glycemic control without adverse effects.

It has been shown that D-allulose is absorbed in the same manner as D-fructose (Hishiike et al., 2013) and is phosphorylated to D-allulose 1-phosphate by ketohexokinase (Raushel and Cleland, 1977). Toyoda and co-workers have demonstrated that D-allulose stimulates translocation of GK using the same mechanism as D-fructose in the liver of both Goto-Kakizaki rats and Wistar rats (control). Furthermore, D-allulose suppressed the increase in plasma glucose levels after glucose loading in Goto-Kakizaki rats (Toyoda et al., 2010). These results indicate that D-allulose uniquely stimulates GK translocation, resulting in increased glucose phosphorylation by GK in the cytoplasm, thereby increasing glycogen storage.

5. Anti-hyperglycemic effects of D-allulose in normal rats

The anti-hyperglycemic effects of D-allulose are summarized as a schematic in Fig. 4. It has been proven that strict glycemic control is associated with a low incidence of both micro- and macro-vascular complications in diabetes, and a delay or inhibition of carbohydrate digestion could be helpful in avoiding postprandial hyperglycemia (Toeller, 1992). Specific inhibitors of α-glucosidase have exhibited a definite therapeutic value in suppressing the postprandial glycemic increase by delaying carbohydrate digestion (Puls et al., 1977). It has been suggested that D-allulose significantly reduces intestinal α-glucosidase and α-amylase activities to 15-25% *in vitro* (Matsuo and Izumori, 2009). In an oral glucose tolerance test (OGTT), D-allulose significantly suppressed the increase of plasma glucose levels in fasted rats after a challenge with 2 g/kg sucrose or maltose in the presence of 0.2 g/kg D-allulose.

Sixty minutes after ingestion, plasma glucose concentrations were significantly lower in the sucrose + D-allulose group than in the sucrose group, with the suppression lasting from 30 to 90 min. At 30, 90, and 120 min after ingestion, the plasma glucose concentration was significantly lower in the maltose + D-allulose group than in the maltose group. Other α-glucosidase inhibitors, such as acarbose, are recognized as potent inhibitors of the activities of intestinal α-glucoamylase, maltase, and sucrase. Furthermore, it has also been shown that acarbose has an inhibitory effect on pancreatic amylase activity (Puls and Keup, 1975; Caspary and Graf, 1979). In many advanced countries, starch accounts for approximately 60% of ingested carbohydrates, with sucrose accounting for 30%, and lactose 10% (Caspary and Graf, 1979). Because the digestion of both starch and sucrose are delayed by D-allulose, as with acarbose, D-allulose can have a valuable effect in reducing postprandial hyperglycemia.

Diurnal variation in glucose levels with two 1-hour feedings per day (8:00 to 9:00 and 20:00 to 21:00), with 5% D-allulose diet, showed lower levels of plasma glucose than regular diet (0% D-allulose) group at every time point throughout the day (Matsuo and Izumori, 2006). Blood glucose levels are regulated by glucose uptake in several peripheral tissues, mostly the liver and skeletal muscles (Iwashita et al., 1996). In addition, insulin secreted from the pancreas stimulates glucose uptake via glucose transporters (GLUTs) (Pedersen et al., 1991). It was suggested that supplemental D-allulose in the diet might reduce postprandial glycemic responses, potentially producing anti-diabetic effects.

The lower plasma glucose levels observed in the D-allulose group might be caused by enhanced glucose uptake in peripheral tissues (Matsuo et al., 1999). We also found that dietary D-allulose increased liver glycogen, but not muscle glycogen (Matsuo and Izumori, 2006), as a large portion of glucose removed from the blood was actively synthesized into

glycogen in the liver and oxidized in skeletal muscles, thus accounting for the absence of increased muscle glycogen (Matsuo and Izumori, 2006). Several other studies in rats reported that plasma glucose levels in the postprandial state, but not in the fasting state, were significantly suppressed by D-allulose compared to other carbohydrates (Matsuo and Izumori, 2006; Matsuo and Izumori, 2009). In our previous study, 4-hr fasting serum glucose levels were significantly lower in the D-allulose group than in the pair-fed control group, although serum insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR), and the quantitative insulin-sensitivity check index (QUICKI) did not differ between groups (Ochiai et al., 2014). Further, serum 1,5-anhydro-D-glucitol (AG), a biochemical parameter for serum glucose control, was significantly higher in the D-allulose group than in the pair-fed control group (Ochiai et al., 2014).

D-Allulose did not cause diarrhea at a dose of 10% of the dietary intake in a rat study (Matsuo et al., 2002b). Although a definite therapeutic value of other known α-glucosidase inhibitors in diabetic patients has been demonstrated, unpleasant side effects associated with incomplete absorption of dietary carbohydrates, such as flatulence, abdominal discomfort, and diarrhea (Toeller, 1992), have been reported. These side effects may be due to the potent inhibition of pancreatic amylases and many intestinal enzymes, which in turn strongly inhibit the digestion of both sucrose and starch. As shown in our previous study, D-allulose mainly inhibited intestinal enzymes and the suppression of amylase was weak, resulting in few adverse effects on the gastrointestinal tract (Matsuo and Izumori, 2009). Moreover, we have already examined the maximum non-effect level of D-allulose in causing diarrhea in humans, which was revealed to be 0.55 g/kg body weight.

6. Anti-hyperglycemic and anti-inflammatory effects of D-allulose in diabetic rats

T2DM is a syndrome characterized by high blood glucose in the context of defective insulin secretion and insulin resistance (Aizawa et al., 1995; Gerich, 1998), precipitating the following adverse consequences: deregulated glucose transport into cells (Choi et al., 2007), decreased glucose utilization by the liver and peripheral tissues, and increased glucose production by the liver (Mevorach et al., 1998). Together, these changes result in the development of insulin resistance. Through several studies, we have shown that D-allulose maintains blood sugar levels uniquely in T2DM model OLETF rats. Initially, we showed that 13 weeks of feeding 5% D-allulose in drinking water maintained blood glucose levels within a normal range in comparison to a control diet (no sugar added) (Hossain et al., 2011). The OGTT after 13 weeks of D-allulose feeding (at 20 weeks of age) also indicated that glucose tolerance was improved with a significant restoration of insulin secretion after glucose loading. In this study, we also showed that translocation of GK in hepatocytes following OGTT was enhanced in the D-allulose-fed group, thus maintaining normal blood glucose levels compared with the control group (Hossain et al., 2011). In a study of chronic 5% D-allulose feeding in OLETF rats, the glucose levels in the control group started to increase slowly at 25 weeks and then sharply increased up to 60 weeks, whereas in the D-allulose group, glucose levels started to increase slightly from 45 weeks and remained constant up to 60 weeks.

Pancreas islet dysfunction is a key characteristic of patients with T2DM that results in hyperglycemia. In the obesity-induced insulin resistant state, pancreatic β-cells initially produce more insulin in an effort to cope with the high insulin demand. This over-activity results in islet hypertrophy that gradually proceeds to β-cell failure (Twombly, 2005), and this progressive failure leads to glucose intolerance followed by T2DM. Insulin resistance initially leads to hyper-insulinemia (Reaven, 1988) followed by hypo-insulinemia when β-cell volume is reduced through injury. In our studies, OLETF rats exhibited initial

hyper-insulinemia peaking at 30 weeks with subsequent hypo-insulinemia, decreasing to near zero levels at week 60, suggesting an initial proliferation of β-cells followed by severe damage. In contrast, plasma insulin levels with D-allulose remained virtually unchanged until week 60 (Hossain et al., 2015). However, one of the important mechanisms for controlling hyperglycemia in T2DM may be the protection of pancreatic β-cells from injury (Gregor and Hotamisligil, 2011) caused by hyperglycemia.

In all our studies, we have observed striking differences in the histological findings pertaining to the morphology of the pancreas islets of Langerhans between D-allulose-fed and control animals (Hossain et al., 2012; Hossain et al., 2015). Briefly, islets of OLETF control rats were observed to be large, disorganized with irregular shapes, and separated into clusters by connective tissue bands, with expansion into adjacent exocrine tissues. As a result, severe loss of islets with extensive fibrosis and fatty degeneration were marked in the hypertrophied islets, in which the degree of fibrosis was assessed using Masson's trichrome stain at 20 weeks (Hossain et al., 2012). To elucidate the islet architecture and β-cell loss in a long-term chronic disease study, we performed both hematoxylin and eosin staining and immunofluorescence staining using anti-insulin and anti-glucagon antibodies. The pattern of distribution of α and β cells (normally insulin-producing β-cells are located centrally and glucagon-producing α-cells peripherally) was disorganized in the control OLETF rats at week 60 (Hossain et al., 2015). The above-mentioned features were prominent in the control rats while islets in the D-allulose-fed rats were well preserved. Thus, a distinct conclusion was that short-term treatment with D-allulose, as well as long-term treatment (up to 60 weeks), exerted beneficial effects against the progression of T2DM and β-cell damage in OLETF rats. During pancreatic injury, pancreatic stellate cells are activated and express the cytoskeletal protein smooth muscle actin for neovascularization as part of the fibrosis process. Alpha smooth muscle actin (α-SMA) immunostaining revealed the presence of multiple intra-islet

proliferative microvessels in control rats, while weak α-SMA staining was observed only in the peri-islet area (not in the islets), suggesting the absence of neovascularization accompanied with fibrosis in D-allulose-treated rats.

Another leading mechanism of insulin resistance is non-resolving low-grade adipocyte inflammation (Gregor and Hotamisligil, 2011) that involves a number of inflammatory cytokines (Moller and Berger, 2003; Koenen et al., 2011), lipids and their metabolites, and reactive oxygen species (ROS) (Dandona et al., 2004). The mechanism involves the accumulation of macrophages in the area of the inflamed adipocytes and subsequent discharge of cytokines such as interleukin 6 (IL-6), IL-1β, and tumour necrosis factor-α (TNF-α) (Bosello and Zamboni, 2000). Treatment with D-allulose significantly suppressed the serum levels of these proinflammatory cytokines, where the most important source of these cytokines is visceral adipose tissue (Moller and Berger, 2003), the aggregation of which was also significantly reduced in the animals treated with D-allulose (Hossain et al., 2015). D-Allulose has also been shown to possess strong scavenging activity towards reactive oxygen species to protect the 6-hydroxydopamine-induced apoptosis in PC12 cells (Takata et al., 2005). Murao et al. has shown the suppression of high glucose-induced expression of monocyte chemoattractant protein 1 (MCP-1) in human vascular endothelial cells (Murao et al., 2007) where MCP-1 is thought to be the major chemotactic factor for monocytes and is found in macrophage-rich areas of atherosclerotic lesions (Schall and Bacon, 1994). A study by Murao et al. indicates that MCP-1 is induced by glucose stimulation, which marks an important link between diabetes mellitus and atherosclerosis that raises the possibility that D-allulose might have therapeutic value in the treatment of atherosclerosis. All these factors, including obesity itself, in T2DM aggravate insulin resistance proportionally to the severity of T2DM (Dandona et al., 2004).

Therefore, it is assumed that the improvement of insulin resistance by D-allulose treatment was facilitated through the control of proinflammatory and inflammatory cytokines in T2DM OLETF rats. However, these findings demonstrated that both short-term and long-term treatment with the rare sugar D-allulose maintains blood glucose levels, lipid profiles with the amelioration of oxidative stress, and proinflammatory cytokines in T2DM rats (Fig. 5).

In a chronic study of D-allulose on T2DM OLETF rats, OGTT was performed at 10, 40, and 60 weeks. The glucose values returned to normal 120 minutes after gavage in all groups, and were close to baseline at week 10; whereas the values were higher and markedly higher in the control group than the D-allulose group at 40 and 60 weeks, respectively (Hossain et al., 2015). It was thus demonstrated that D-allulose exhibits potent anti-hyperglycemic activity. It is also noted that D-allulose does not cause hypoglycemia either in normal, pre-diabetic, or diabetic rats.

7. Anti-hyperlipidemic effects of D-allulose

Uncontrolled intake of sugar-sweetened beverages, like sucrose or corn syrup (both include D-fructose), has been the focus of investigation as one of the causes of obesity (Schulze et al., 2004; Malik et al., 2006). Because D-fructose is known to be lipogenic and related to the development of metabolic syndrome (Rutledge and Adeli, 2007), D-allulose, an epimer of D-fructose, may also be lipogenic. However, as mentioned below, D-allulose has anti-hyperlipidemic and anti-obesity potential. We have reported that ≈70% of D-allulose was absorbed and excreted via urine (Tsukamoto et al., 2014) and a small unabsorbed portion was not fermented in either rats (Matsuo et al., 2003) or humans (Iida et al., 2010), indicating the non-caloric value of D-allulose. Thus, D-allulose is likely to be inert in terms of energy metabolism.

However, several studies have reported the anti-obesity activity of D-allulose through decreasing adipose tissue weight in animals (Matsuo et al., 2001b; Matsuo and Izumori,

2006; Yagi and Matsuo, 2009; Hossain et al., 2011; Chung et al., 2012; Hossain et al., 2012; Ochiai et al., 2013; Ochiai et al., 2014) and humans (Iida et al., 2013). Because obesity is known to cause insulin resistance, leading to T2DM, D-allulose could be useful in maintaining a healthy body weight and preventing the onset of diabetes. In rats, 3% D-allulose feeding for 18 months significantly decreased abdominal fat and body weight compared to sucrose, while no difference in energy intake was found (Yagi and Matsuo, 2009). In another study, 5% D-allulose in the diet lowered the overall carcass fat content, and specifically reduced the abdominal adipose tissue weight with no difference in body weight gain in comparison to sucrose and D-fructose (Matsuo and Izumori, 2006). In T2DM model OLETF rats, 5% D-allulose in the drinking water significantly decreased both abdominal fat weight and body fat mass compared to control animals (Hossain et al., 2011). Furthermore, when high-fat diet-induced obese rats were given a normal diet supplemented with different doses of D-allulose, body weight gain and fat accumulation in the body were dose-dependently reduced compared to rats fed a normal diet without D-allulose (Chung et al., 2012). In a mechanistic investigation, Chung et al. showed that D-allulose inhibited the differentiation of mesenchymal stromal cells derived from C57BL/6J mice into adipocytes in a dose-dependent manner. In addition, low numbers of oil red-stained lipid droplets in the liver were observed in the sugar-fed rats compared to their control counterparts (Hossain et al., 2011). Furthermore, Hossain et al. showed decreased adipocyte size in OLETF rats fed D-allulose compared to controls (Hossain et al., 2012).

Collectively, D-allulose appears to have anti-obesity potential, which is perhaps due to diminished food intake (Hossain et al., 2011; Chung et al., 2012; Hossain et al., 2012; Iida et al., 2013; Ochiai et al., 2013; Ochiai et al., 2014). Diminished body weight and lowered carcass fat have also been observed when rats were pair-fed high-sucrose diets with or without D-allulose (Ochiai et al., 2014). This indicates that the anti-obesity effects are not

simply due to decreased food intake, but involve other mechanisms that are responsible for the decreased adipose tissue weight and fat mass. A potential mechanism that could account for these observations is enhanced 24-hr energy expenditure with a 5% D-allulose diet (Ochiai et al., 2014), with enhanced fat oxidation and reduced carbohydrate oxidation (Nagata et al., 2015) compared to a control diet.

Regarding the anti-hypertriglyceridemic action of D-allulose, although no clear-cut evidence has been shown, a variety of observations have been made by several authors in our group. Matsuo et al. reported lower TG levels 8 weeks after feeding a 5% D-allulose diet compared to a control diet (Matsuo and Izumori, 2006). Subsequently, the same group reported that there was no difference in serum TG levels between rats fed diets with or without D-allulose for 12 months (Yagi and Matsuo, 2009). However, Hossain et al. showed decreased TG levels in OLETF rats with 5% D-allulose feeding. On the other hand, Nagata et al. reported somewhat higher TG levels with D-allulose, without a significant difference compared to controls, while hepatic TG levels tended to be lower than in controls (Nagata et al., 2015). In addition, plasma TG levels in *db/db* mice fed D-allulose were not different from animals fed the control diet for 28 days, while hepatic TG was significantly decreased (Baek et al., 2010). Clinically, a similar effect was observed when D-allulose was fed as a mixture with other sugars (Iida et al., 2013). The reported mechanism involves decreased activities of lipogenic enzymes in the liver (fatty acid synthase and glucose-6-phosphate dehydrogenase) with diets containing 5% D-allulose with glucose or fructose (Matsuo et al., 2001a) or a high-sucrose diet with 5% D-allulose compared to the control sucrose-based diet (Ochiai et al., 2014). One study showed elevated levels of lipoprotein lipase (LPL) in the soleus muscle of rats fed 5% D-allulose compared to D-fructose (Matsuo et al., 2001b), while the same group showed unaltered LPL activity in another study (Matsuo et al., 2001a; Ochiai et al., 2014). When LPL

activity was found to be activated by the D-allulose diet (compared to the control diet), serum TG levels were observed to be significantly higher (Ochiai et al., 2014). Furthermore, LPL activity in heart and adipose tissues was not influenced by D-allulose (Matsuo et al., 2001a; Ochiai et al., 2014). Thus, although LPL is involved in TG hydrolysis in lipoproteins, D-allulose is unlikely to affect TG levels thorough altering LPL activity. Nagata et al. showed that 3% D-allulose decreased gene expression of cluster of differentiation 36 (CD-36), scavenger receptor class B type I (SR-B1), and microsomal TG transfer protein (MTP) in the small intestine, all of which are associated with lipid absorption, indicating that D-allulose modulates lipid processing at the small intestinal level and produces a hypolipidemic effect (Nagata et al., 2015). Taken together, although the anti-hypertriglyceridemic action of D-allulose remains unclear at present, it is possible that D-allulose alters the activities of lipogenic and lipolytic enzymes, thus altering TG metabolism. However, further studies are required to clarify the underlying mechanism of D-allulose's anti-hypertriglyceridemic action. With regard to cholesterol metabolism, serum cholesterol was significantly decreased by D-allulose feeding compared to a control diet (Ochiai et al., 2014; Nagata et al., 2015). In contrast, plasma cholesterol levels in *db/db* mice fed D-allulose were not different from those in animals fed a control diet for 28 days (Baek et al., 2010). In diet-induced obese rats, D-allulose feeding resulted in higher serum cholesterol levels compared to the AIN-93M-based control diet, without significantly changing TG levels (Chung et al., 2012). Nagata et al. did not find a significant effect of D-allulose on gene expression of hepatic 3-hydroxyl-3-methylglutaryl-coenzyme A, which is essential for cholesterol synthesis (Nagata et al., 2015). Presently, no concrete data are available on the relationship between D-allulose and cholesterol metabolism. However, to clarify the anti-hyperlipidemic action of D-allulose, further studies on factors affecting lipid metabolism (absorption, synthesis, transport, uptake, and clearance) are warranted.

8. Clinical trials of D-allulose on normal and borderline diabetic people

The rapidly increasing incidence of obesity, obesity-induced T2DM, and the cost of managing the chronic complications of these disorders are becoming an enormous problem in the modern world. Therefore, it is of prime importance to look for effective therapeutic interventions for both the prevention and treatment of diabetes and its complications (Takeuchi et al., 1996). The rare sugar D-allulose has attracted much attention for its promising anti-hyperglycemic and anti-hyperlipidemic effects in experimental studies (Matsuo et al., 1999; Matsuo and Izumori, 2006; Matsuo and Izumori, 2009; Hossain et al., 2011; Hossain et al., 2012; Hossain et al., 2015).

Based on these findings, and before proceeding to therapeutic use on obese and diabetic patients, our research group has conducted a trial on healthy subjects and reported that 5 g or more D-allulose significantly suppressed blood glucose elevation following ingestion of 75 g maltodextrin (carbohydrate) (Iida et al., 2008), as maltodextrin is used as the carbohydrate source in the OGTT for the diagnosis of diabetes in Japan. The study showed that a ratio of approximately 1:15 (D-allulose:carbohydrate) was effective in suppressing glucose elevation. It was also confirmed that a single ingestion of only 5 g D-allulose had no effect on blood glucose and insulin levels, meaning that hypoglycemia is not be induced by D-allulose.

However, from the viewpoint of diabetes prevention, it is important to prove the effect of D-allulose in subjects with impaired glucose tolerance. Hence, we conducted another study in borderline diabetic subjects who were given 5 g D-allulose with a standard meal in the first week, followed by another 5 g D-allulose with the same standard meal 1 week later. Subjects underwent overnight fasting and the following morning fasting blood sugar was measured. Subjects were then provided with a normal meal (425 kcal with 84.5 g of carbohydrate, 13.3 g of protein, and 3.7 g of fat) with 200 ml tea (with or without 5 g of D-allulose) and blood samples were collected 30, 60, 90, and 120 min after the meal. D-Allulose decreased blood

glucose levels significantly at 30 and 60 min after meal ingestion compared to control subjects, although there was no significant difference in serum insulin levels between the groups (Hayashi et al., 2010).

Moreover, we have performed several clinical trials on both normal and diabetic volunteer subjects and found that D-allulose dose-dependently suppresses blood glucose levels after glucose loads in OGTT (unpublished data). Overall, the control of postprandial glucose levels is necessary, and a number of studies have mentioned the importance of dietary factors that can suppress postprandial blood glucose elevations (Jenkins et al., 1982; Wolever et al., 1985; Brand-Miller et al., 2007). It is thus suggested that D-allulose-supplemented diets might be appropriate physiological tools to prevent obesity and T2DM.

The mechanism underlying the ability of D-allulose to control high blood glucose levels is described elsewhere in this review (summarized in Fig. 4). The suppressive effect of D-allulose on the elevation of plasma glucose concentrations in rats following carbohydrate administration is attributable to the inhibition of α-glucosidase and enhanced translocation of GK. Thus, a similar suppressive mechanism in humans is expected. The safety of D-allulose was evaluated in subjects with normal blood glucose levels (Hayashi et al., 2010) through a meal-loading study where D-allulose or D-glucose was given with meals three times a day for 12 continuous weeks. The safety parameters were assessed from blood analysis, urine analyses, physical examinations, and subjective symptoms, such a flatulence, diarrhea or loose stools, constipation, and distension. The results revealed that serum levels of cholinesterase (CHE) and lactate dehydrogenase (LDH) were within normal ranges in the test group; furthermore, no alterations in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (γ-GTP) were identified, suggesting normal hepatic function. Similarly, carbohydrate metabolism indicators (blood glucose, insulin, haemoglobin A1c (HbA1c), and glycoalbumin levels were unchanged.

Overall, abnormal findings and clinical disorders were not observed following long-term ingestion of 5 g D-allulose three times a day (Hayashi et al., 2010).

9. Conclusion

D-Allulose is a rare sugar with almost zero calories, sweetness that is 70% of sucrose, with some cooling sensation and no bitterness. Trace amounts of D-allulose are found naturally in the leaves of *Itea*, a plant found in Southeast Asia including Japan. Presently, a large amount of D-allulose is being produced through enzymatic methods discovered by a research group at Kagawa University, Japan. In this review, we focused mainly the physiological functions of D-allulose with a view to controlling caloric intake through carbohydrate and lipid metabolism, thereby decreasing the prevalence of obesity and T2DM.

The onset of lifestyle-related diseases (non-communicable diseases) is closely related to overconsumption of calories, especially carbohydrates and sugar-related sweeteners. In addition to high-caloric, sugar-related sweeteners, consumption of the most widely used non-caloric artificial sweeteners (NAS) has been shown to increase body weight gain followed by increased risk of T2DM (Horwitz et al., 1988). In March 2014, the World Health Organization (WHO) launched a new guideline on the intake of sugars. The WHO's current recommendation is that daily sugar intake should be less than 10% of total daily energy intake. The WHO further suggested that daily intake shall be reduced to less than 5% for additional benefits, which is equivalent to about 25 g for an adult with normal body mass index (BMI). However, this recommendation is difficult to realize even in Japan, which is a country with one of the lowest rates of sugar intake, with people typically consuming an average of 50 g per day. Consumption of sugar and high fructose corn syrup (HFCS) through sugar-sweetened beverages and processed foods has been increasing and is said to be responsible for the increased rate of obesity and T2DM. The replacement of sugar, HFCS, and NAS with the enzymatically prepared natural sugar D-allulose is therefore an attractive method for reducing

the total amount of sugar intake, as D-allulose is a zero-calorie sweetener that has additional functions for lowering glucose levels and inhibiting fat accumulation. Even if the total sugar and HFCS intake remain the same, we can enjoy healthier foods and subsequently healthier lives with a lower risk of developing diabetes mellitus and obesity, as D-allulose has been shown to inhibit sucrase activity and the subsequent absorption of D-glucose and D-fructose through the gut wall. Carbohydrate metabolism and lipid metabolism are closely related and impairment of either immediately affects the other. It is therefore theoretically important to concomitantly control both metabolic processes, and D-allulose is one of the rare candidates that exhibit such effects.

Hypothetically, we can classify sugar generations according to their physiological importance and adverse effects (Table 1). The first generation of sweeteners includes sucrose (regular sugar) and HFCS, which have the maximum quality of sweetness with high calories and are highly affordable. However, these sweeteners have given rise to problems such as obesity, T2DM, dental caries, and metabolic syndrome. The second generation sweeteners are high-intensity sweeteners such as aspartame, sucralose, and saccharin. Many of these sweeteners have zero calories and can replace sugar and HFCS to significantly reduce caloric intake from sweeteners. However, there are some reports that describe adverse effects associated with these artificial sweeteners, and cautions have been raised for the usage of these sweeteners (Suez et al., 2014; Swithers, 2013). Consumers feel that these high-intensity sweeteners have different sweetness as compared to sugar. The third generation sweeteners are sugar alcohols, such as xylitol (2.4 kcal/g) and erythritol (0.2 kcal/g). They are used as low-calorie sweeteners and xylitol has been used as an anti-cariogenic sweetener for dental health. However, their laxative effects can create difficulty in gaining wide applicability. It is also noted that these sugar alcohols have some lingering taste. D-Allulose may be classified with fourth- generation sweeteners, which are natural sweeteners with low or zero calories and

with sugar-like taste, and have additional functions. If we consume only first-generation sweeteners, we tend to have a higher risk of developing lifestyle-related diseases. The second- and third-generation sweeteners have therefore been developed and recommended for use in combination with sugars and HFCS. The new fourth-generation sweeteners, including D-allulose, are similar to the second- and third-generation sweeteners in the sense that their use will reduce energy intake when used as sugar substitutes. They are different in the sense of having various functions without producing adverse effects. Notably, D-allulose has been designated as GRAS by the US-FDA. We do not have to completely eliminate first-generation sweeteners from our diets, and recommend using D-allulose at a minimum of 5% - 10% of total sugar use. In the near term, the mass production of D-allulose will make the cost of this sugar reasonably cheap, and hopefully its good taste and health benefits will become widely available.

Recently, we have been rapidly developing a research program aimed at identifying the physiological functions of rare sugars other than D-allulose. For example, the anti-oxidative and anti-cancer effects of D-allose (Yamaguchi et al., 2008; Nakamura et al., 2011) and the anti-caries effect of D-tagatose (Sawada et al., 2015) are being examined. As such, by promoting the use of rare sugars we may be able to offer numerous beneficial functions to prevent lifestyle-related diseases in the near future, as well as to effectively solve the problems created by excess sugar intake.

10. Figure Legends

Figure 1: Structures and enzymatic conversion of D-glucose, D-fructose, D-allulose, and D-allose. Inter-conversion between D-glucose, D-fructose, D-allulose, and D-allose is

catalyzed by the following enzymes: a, xylose isomerase; b, D-tagatose 3-epimerase, c, L-rhamnose isomerase. Reactions catalyzed by D-tagatose 3-epimerase can link ketohexoses by epimerization at the C-3 position.

Figure 2: The transport route of sugars (D-glucose, D-fructose, and D-allulose) from the intestinal lumen to the lamina propria across the human intestinal epithelial cell layer or Caco-2 cell layer. Typically, the uptake of glucose and fructose in the enterocyte brush border is mediated by SGLT1 and GLUT5, respectively, while efflux from the enterocyte into the portal circulation is mediated by GLUT2. Interestingly, D-allulose uptake is mediated by the fructose transporter GLUT5 and effluxed by the transporter GLUT2. It has been determined that the absorption rate of D-allulose is low in comparison to D-glucose and D-fructose, which in turn helps to lower blood glucose levels. This process is described briefly in section 3. SGLT1, sodium-dependent glucose transporter 1; GLUT5, glucose transporter 5; GLUT2, glucose transporter 2; GLUT7, glucose transporter 7.

Figure 3: Regulatory mechanism of hepatic glucose metabolism by the nucleocytoplasmic translocation of GK. D-Allulose-induces GK translocation from the nucleus to the cytoplasm. In the postabsorptive state (blue arrows), GK exists predominantly in the nucleus in association with GKRP with the majority of GK in an inactive state. Glucose is produced through glucose-6-phosphatase in the endoplasmic reticulum and is not phosphorylated. In the postprandial state (black arrows), D-glucose and D-fructose enter the hepatocytes, and the latter hexose is converted to D-fructose 1-phosphate by ketohexokinase (KHK). Both D-fructose 1-phosphate and D-glucose promote the release of GK from the regulatory protein. The free form of GK then translocates into the cytoplasm and catalyzes the phosphorylation of D-glucose. Thus, rapid glucose utilization takes place under these conditions. GKRP acts as an anchor for GK in the nucleus, and a regulator of GK translocation within hepatocytes.

Like D-fructose, D-allulose (red arrows) is also converted to D-allulose 1-phosphate by KHK. This phosphate ester binds to GKRP, resulting in GK being released from GKRP, thereby increasing glucose utilization in the liver. A1P, D-allulose 1-phosphate; F1P, D-fructose 1-phosphate; F6P, D-fructose 6-phosphate; G6Pase, D-glucose-6-phosphatase; GK, glucokinase; GKRP, glucokinase regulatory protein; G1P, D-glucose 1-phosphate; G6P, D-glucose 6-phosphate; KHK, ketohexokinase.

Figure 4: Multiple anti-hyperglycemic actions of D-allulose have been identified. When polysaccharides are consumed, such as starch and sugars, they are digested into monosaccharides in the gastrointestinal (GI) tract by various digestive enzymes including α-amylase, α-glucosidase, maltase, and sucrase. D-Allulose mildly inhibits these enzymes and the production of monosaccharides, such as D-glucose and D-fructose, is reduced. D-Allulose also inhibits the absorption of D-glucose via transporters in the intestinal mucosa, resulting in reduced amounts of D-glucose transported into the blood. Because D-allulose is a monosaccharide, it is absorbed through the GLUT5 transporter in the intestine. When D-allulose arrives at the liver, it stimulates glucokinase (GK) translocation from the nucleus to the cytosol, resulting in GK activation and the stimulation of glycogen synthesis in the liver. D-Allulose, together with reduced serum glucose levels, has a beneficial effect on pancreatic β-cell function. Taken together, D-allulose functions as an anti-hyperglycemic sweetener.

Figure 5: Schematic of anti-diabetes and anti-obesity actions of D-allulose. D-Allulose has shown unique characteristics in preventing obesity and T2DM through multiple pathways. In situations where obesity leads to T2DM, adipose tissue-mass enlargement occurs with the infiltration of inflammatory macrophages, and consequently the release of inflammatory cytokines from both macrophages and adipocytes. Additionally, in the hyperglycemic state,

pancreatic β-cells are forced to produce more insulin to minimize the elevated blood glucose levels, thereby resulting in islet hypertrophy that gradually proceeds to β-cell failure and subsequent glucose intolerance and T2DM. Moreover, glucose output from the liver is increased and glucose utilization by adipose tissues and skeletal muscles is reduced. However, the hyperglycemia-lowering effect of D-allulose is mediated by suppressing pro-inflammatory adipocytokines released from inflamed adipocytes, thereby protecting pancreatic islet cells from hyperglycemia-induced injury, enabling the resumption of insulin production, decreasing intestinal glucose absorption, and enhancing glucose uptake by adipose and muscle tissues.

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Table 1 Generation of sweeteners

Generation	Sugars	Characteristics
1st	Sucrose, glucose, fructose, maltose, lactose and high fructose corn syrup	☐ Source of calorie intake ☐ Sugar or sugar-like taste ■ Overuse causes lifestyle-related diseases such as obesity and diabetes
2nd	High-intensity sweeteners or non-caloric artificial sweeteners, such as aspartame, sucralose, saccharin etc.	☐ Reduction of calorie intake ■ Unlike sugar sweet ■ Some adverse effects such as aggravation of obesity and diabetes
3rd	Sugar alcohols such as xylitol, erythritol, sorbitol, maltitol etc.	☐ Reduction of calorie intake ■ Some lingering taste ■ Some sugar alcohols have a laxative effect
4th	Natural low-/zero-calorie sweeteners with functions such as rare sugars	☐ Reduction of calorie intake ☐ Sugar-like taste ☐ Additional functions ☐ Few/no adverse effects

Sweeteners are classified into 4 generations.

The advantages (open squares) and disadvantages (closed squares) are described.

Figure 1

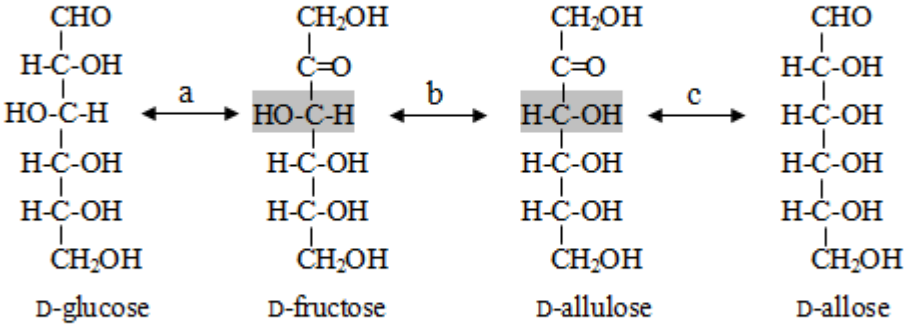

Figure 2

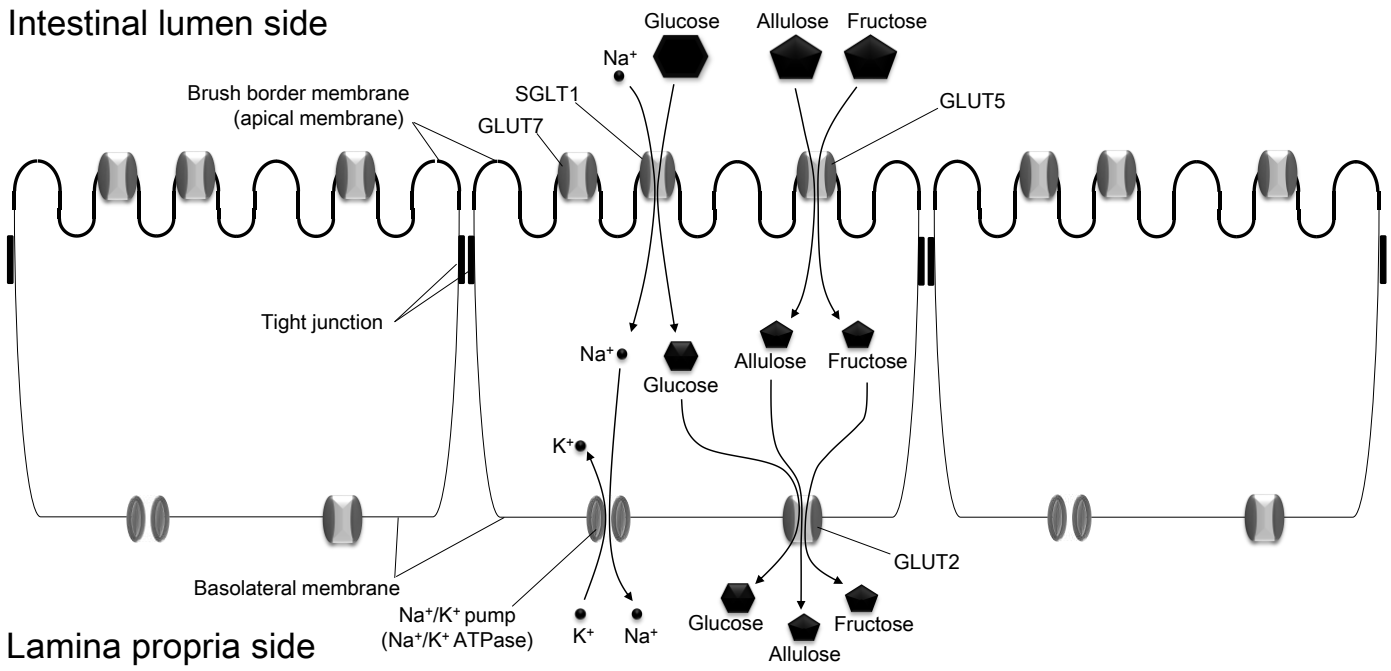

Figure 3

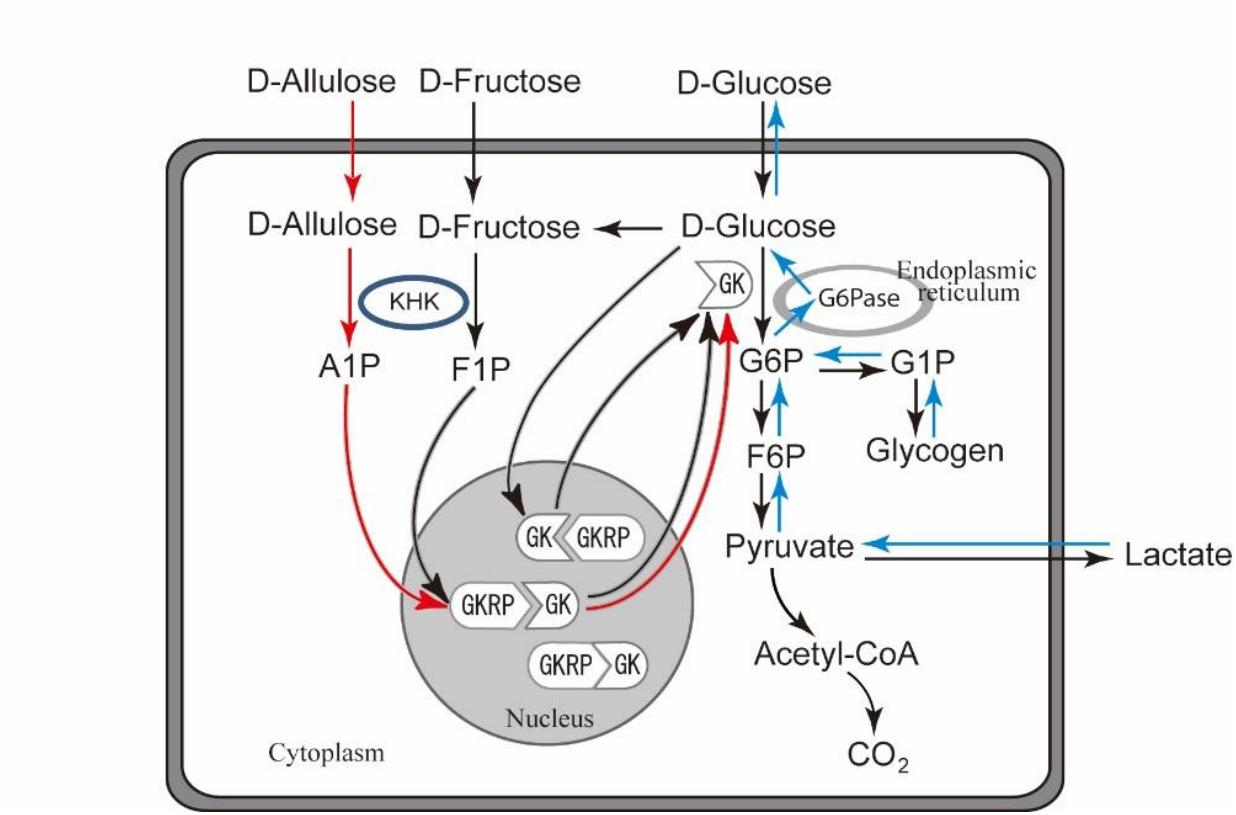

Figure 4

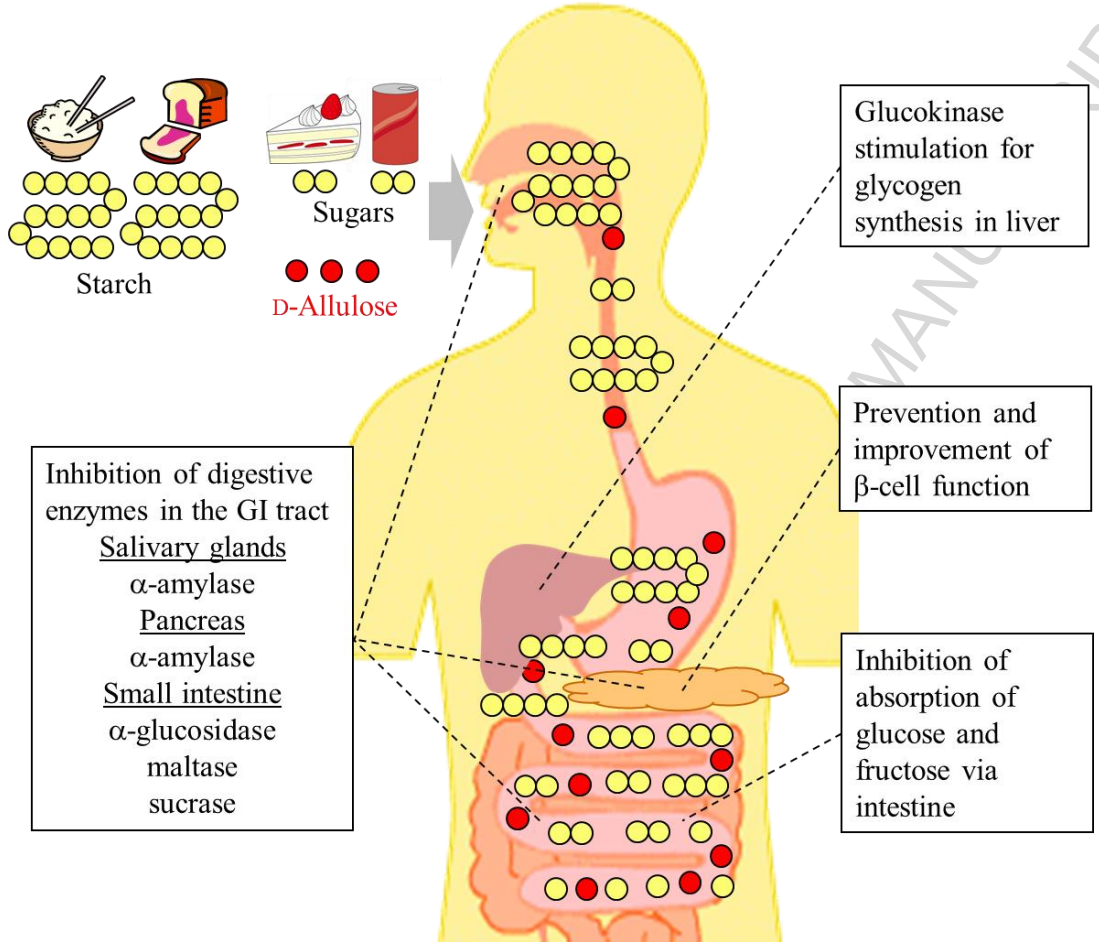

Figure 5